CLAIMS

1. A method for synthesizing a polynucleotide of a predetermined sequence, which method comprises the steps of:

(a) providing an initiating substrate comprising a nucleoside having an unprotected 3'-hydroxyl group; and

(b) reacting under enzymatic conditions in the presence of a catalytic amount of an enzyme said 3'-hydroxyl group of said initiating substrate with a nucleoside 5'-triphosphate having a removable blocking moiety protecting the 3' position of said nucleoside 5'-triphosphate and selected according to the order of said predetermined sequence, whereby said enzyme catalyzes the formation of a 5' to 3' phosphodiester linkage between said unprotected 3'-hydroxyl group of said initiating substrate and the 5'-phosphate of said nucleoside 5'-triphosphate to produce said polynucleotide.

2. The method of claim 1 further comprising the step of:

(c) removing the blocking moiety protecting the 3' position of said nucleoside 5'-triphosphate to produce an initiating substrate having an unprotected 3'-hydroxyl group.

3. The method of claim 2 further comprising the step of repeating steps (b) and (c) at least once.

4. The method of claim 2 further comprising

repeating the steps (b) and (c) until the polynucleotide having the predetermined sequence is obtained.

5. The method of claim 1, wherein said initiating substrate is selected from the group consisting of ribonucleosides, deoxynucleosides, nucleotides, and single and double stranded oligonucleotides and polynucleotides.

6. The method of claim 1, wherein said initiating substrate further comprises oligonucleotide sequences.

7. The method of claim 6, wherein said oligonucleotide sequences are attached to non-nucleoside molecules.

8. The method of claim 1, wherein said initiating substrate is immobilized on a solid support.

9. The method of claim 8, wherein said solid support is selected from the group consisting of cellulose, Sepharose®, controlled-pore glass, silica, Fractosil®, polystyrene, styrene divinyl benzene, agarose, and crosslinked agarose.

10. The method of claim 1, wherein said enzyme is a template-independent polynucleotide polymerase.

11. The method of claim 10, wherein said template-independent polynucleotide polymerase is terminal deoxynucleotidyl transferase.

12. The method of claim 1, wherein said removable blocking moiety is removed in under 10 minutes to produce a hydroxyl group at the 3' position of the 3'-terminal nucleoside.

13. The method of claim 12, wherein said removable blocking moiety is removed in under 2 minutes to produce a hydroxyl group at the 3' position of the 3'-terminal nucleoside.

14. The method of claim 1, wherein said removable blocking moiety is selected from the group consisting of esters, ethers, carbonitriles, phosphates, phosphoramide carbonates, carbamates, borates, nitrates, sugars, phosphoramidates, phenylsulfenates, sulfates, and sulfones, wherein said removable blocking moiety is linked to the 3' carbon of said nucleoside 5'-triphosphate.

15. The method of claim 14, wherein said removable blocking moiety is selected from the group consisting of an ester, a phosphorous containing moiety, and an ether.

16. The method of claim 15, wherein said ester is selected from the group consisting of toluoyl ester, isovaleroyl ester, benzoyl ester, 4-nitrobenzoyl ester, 2,6 dimethylbenzoyl ester, 3,5 dimethylbenzoyl ester and dimethylbenzoyl ester.

17. The method of claim 15, wherein said ether is selected from the group consisting of bis(2-chloroethoxy)methyl ether, 4-methoxytetrahydropyranyl ether, tetrahydrafuranyl ether, 1-ethoxyethyl ether, tri(p-methoxyphenyl)methyl ether, di(p-methoxy)phenylmethyl ether, t-butyldimethylsilyl ether.

18. The method of claim 15, wherein said phosphorous containing moiety is selected from the group consisting of phosphate, phosphoramidate and phosphoramide.

19. The method of claim 1, further comprising the

step of treating said nucleoside 5'-triphosphate having said removable blocking moiety with deblocking solution whereby said removable blocking moiety is removed.

20. The method of claim 19, wherein said deblocking solution comprises a divalent cation.

21. The method of claim ~~19~~ 20, wherein said divalent cation is Co^{++}.

22. The method of claim 19, wherein said deblocking solution comprises a buffer selected from the group consisting of dimethylarsinic acid, tris[hydroxymethyl] amino methane, and 3-[m-morpholine] propianosulphonic acid.

23. The method of claim 19, wherein said deblocking solution comprises an enzyme capable of removing said removable blocking moiety.

24. The method of claim 19, wherein said treating occurs in under 10 minutes.

25. The method of claim ~~19~~ 24, wherein said treating occurs in under 2 minutes.

26. The method of claim 1, wherein said removable blocking moiety is linked to a solid support.

27. The method of claim 26, further comprising the step of cleaving said polynucleotide from said solid support.

28. The method of claim 27, wherein said cleaving produces a polynucleotide having a 3'-hydroxyl group at

its 3' terminus.

29. The method of claim 26, wherein said removable blocking moiety linked to said solid support is selected from the group consisting of esters, ethers, carbonitriles, phosphates, carbonates, carbamates, borates, nitrates, sugars, phosphoramidates, phenylsulfenates, sulfates, sulfones and amino acids, wherein said removable blocking moiety is linked to the 3' position of said nucleoside 5'-triphosphate and is also linked to said solid support.

30. A polynucleotide having a predetermined sequence produced using the method of claims 1-29.

31. A polynucleotide having a predetermined sequence of greater than five nucleotides produced using the method of claims 1-29.

32. A composition of matter comprising:

(a) a catalytic amount of a template independent enzyme; and

(b) nucleoside 5'-triphosphate having a removable blocking moiety protecting the 3' position of said nucleoside 5'-triphosphate.

33. The composition of claim 32, further comprising an initiating substrate comprising a nucleoside having an unprotected 3'-hydroxyl group.

34. The composition of claim 33, wherein said catalytic amount of enzyme catalyzes the formation of a 5' to 3' phosphodiester linkage between 99 percent of said unprotected 3'-hydroxyl group of said initiating substrate and the 5'-phosphate of said nucleoside 5'-triphosphate

within 10 minutes.

35. The composition of claim 33, wherein said catalytic amount of enzyme catalyzes the formation of a 5' to 3' phosphodiester linkage between 99 percent of said unprotected 3'-hydroxyl group of said initiating substrate and the 5'-phosphate of said nucleoside 5'-triphosphate within 2 minutes.

36. The composition of claim 33, wherein the concentration of said initiating substrate is between 1 nmol. and 100 mmol.

37. The composition of claim 32, wherein the concentration of said nucleoside 5'-triphosphate is from 10 μmol. to 1 mmol.

38. The composition of claim 32, wherein said nucleoside 5'-triphosphate is present at a concentration of ten times the Km of said enzyme.